Incidence and risk factors for new-onset diabetes in HIV-infected patients: the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study

De Wit, S ; Sabin, C A ; Weber, R ; Worm, S W ; Reiss, P ; Cazanave, C ; El-Sadr, W ; D'Arminio Monforte, A ; Fontas, E ; Law, M G ; Friis-Møller, N ; Phillips, A

Abstract: OBJECTIVE: The aims of this study were to determine the incidence of diabetes among HIV-infected patients in the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) cohort, to identify demographic, HIV-related, and combination antiretroviral therapy (cART)-related factors associated with the onset of diabetes, and to identify possible mechanisms for any relationships found. RESEARCH DESIGN AND METHODS: D:A:D is a prospective observational study of 33,389 HIV-infected patients; diabetes is a study end point. Poisson regression models were used to assess the relation between diabetes and exposure to cART after adjusting for known risk factors for diabetes, CD4 count, lipids, and lipodystrophy. RESULTS: Over 130,151 person-years of follow-up (PYFU), diabetes was diagnosed in 744 patients (incidence rate of 5.72 per 1,000 PYFU [95% CI 5.31-6.13]). The incidence of diabetes increased with cumulative exposure to cART, an association that remained significant after adjustment for potential risk factors for diabetes. The strongest relationship with diabetes was exposure to stavudine; exposures to zidovudine and didanosine were also associated with an increased risk of diabetes. Time-updated measurements of total cholesterol, HDL cholesterol, and triglycerides were all associated with diabetes. Adjusting for each of these variables separately reduced the relationship between cART and diabetes slightly. Although lipodystrophy was significantly associated with diabetes, adjustment for this did not modify the relationship between cART and diabetes. CONCLUSION: Stavudine and zidovudine are significantly associated with diabetes after adjustment for risk factors for diabetes and lipids. Adjustment for lipodystrophy did not modify the relationship, suggesting that the two thymidine analogs probably directly contribute to insulin resistance, potentially through mitochondrial toxicity.

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Incidence and risk factors for new onset diabetes mellitus in HIV infected patients: the D:A:D study

Stephane De Wit, M.D., Ph.D. (1), Caroline A. Sabin, Ph.D.(2), Rainer Weber, M.D.(3), Signe Westring Worm (4), Peter Reiss, M.D., Ph.D. (5), Charles Cazanave, M.D.(6), Wafaa El-Sadr, M.D., M.P.H.(7), Antonella d'Arminio Monforte, M.D., D.M.Sc.(8) , Eric Fontas, M.D.(9), Matthew G. Law, Ph.D.(10),
Nina Friis-Møller, M.D., Ph.D.(4), Jens D. Lundgren, M.D., D.M.Sc. (4)

(1) Centre Hospitalier Universitaire Saint-Pierre, Brussels
(2) Royal Free and University College, London
(3) University Hospital Zurich, Zurich, Switzerland
(4) University of Copenhagen, Copenhagen
(5) Academic Medical Center, Amsterdam
(6) Bordeaux 2 University, Bordeaux, France
(7) Columbia University, Harlem Hospital, New York
(8) University of Milan, Milan
(9) Centre Hospitalier Universitaire Nice, Hôpital de l'Archet, Nice, France
(10) National Centre in HIV Epidemiology and Clinical Research, Sydney

Running Title: Diabetes and antiretroviral therapy

Corresponding Author :
Stéphane De Wit, MD, PhD
Department of Infectious Diseases
St Pierre University Hospital
322, rue Haute
B- 1000 Brussels Belgium.
stephane_dewit@stpierre-bru.be

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ABSTRACT

Objective: To determine the incidence of Diabetes Mellitus (DM) among HIV patients in the D:A:D (Data Collection on Adverse Events of Anti-HIV Drugs) cohort, to identify demographic, HIV-related and combination antiretroviral therapy (cART)-related factors associated with the onset of DM, and to identify possible mechanisms for any relationships found.

Research Design and Methods: D:A:D is a prospective observational study of 33,389 HIV patients; DM is a study endpoint. Poisson regression models assessed the relation between DM and exposure to cART after adjusting for known risk factors for DM ,CD4 count, lipids and lipodystrophy.

Results: Over 130,151 person-years of follow-up, 744 patients were diagnosed with DM (incidence rate of 5.72 per 1,000 PYFU (95% CI: 5.31-6.13)). The incidence of DM increased with cumulative exposure to cART , association that remained significant after adjustment for potential risk factors for DM. The strongest relationship with DM was exposure to stavudine; exposure to zidovudine and didanosine were also associated with an increased risk of DM. Time-updated measurements of total cholesterol, HDL-cholesterol and triglycerides were all associated with DM. Adjusting for each of these variables separately reduced slightly the relationship between cART and DM. While lipodystrophy was significantly associated with DM, adjustment for this did not modify the relationship between cART and DM.

Conclusion: Stavudine and zidovudine are significantly associated with DM after adjustment for risk factors for DM and lipids. Adjustment for lipodystrophy did not modify the relationship, suggesting the two thymidine analogues probably directly contribute to insulin resistance potentially through mitochondrial toxicity.

Mortality and morbidity from HIV and its complications have dramatically declined since the advent of combination anti-retroviral therapy (c-ART). However, metabolic disorders have emerged - impaired glucose tolerance and diabetes, as well as lipid disorders - leading to an increase of cardiovascular disease.

Data from the D:A:D (Data Collection on Adverse Events of Anti-HIV Drugs) Study suggest that the risk of a myocardial infarction is more than doubled among HIV patients with Diabetes Mellitus (DM) (1). I Insulin resistance among treated HIV-infected patients is multifactorial: in addition to the common contributors to insulin resistance (e.g. obesity, genetic influences, physical inactivity), antiretroviral drugs and lipodystrophy (which may be a consequence of treatment, particularly with thymidine analogues), are involved.

The aims of this analysis were to estimate the incidence of new onset DM among patients with no history of DM at entry to D:A:D, to identify demographic, HIV-related and cART -related factors that were associated with the onset of DM, and to identify possible mechanisms for any relationships found.

RESEARCH DESIGN AND METHODS

The D:A:D study is a large, prospective observational study formed by the collaboration of 11 cohorts of HIV-infected patients. The primary aim of the study was to establish whether an association exists between the use of cART and an increased risk of CVD. The 11 cohorts currently contribute data on 33,389 HIV-positive patients followed at 212 clinics in Europe, the US, Argentina and Australia. The D:A:D study methodology has been described in detail (1). Patients eligible for inclusion were all being actively followed up at the time of initiation of the D:A:D protocol , irrespective of antiretroviral treatment status. Patients were followed prospectively, and data were obtained during visits scheduled as part of regular medical care. Patient follow-up started between December 1999 and April 2001 (D:A:D Phase I) and April 2001 and January 2005 (D:A:D Phase II). At enrolment, and at least every 8 months thereafter, standardized data collection forms were completed, including socio-demographic characteristics, clinical data (AIDS events and known risk factors for CVD), laboratory markers (CD4 cell counts, HIV RNA load, and total cholesterol, HDL-cholesterol and triglyceride levels), and treatment variables (antiretroviral treatment and drugs modifying lipid levels or risk of CVD). Use of ritonavir includes both full and boosting doses. Data are transformed into a standardized format, transferred to the coordinating center (Copenhagen HIV Programme, Hvidovre Hospital, Denmark) as anonymised computerized files, and merged into a central dataset.

Endpoint definition. DM has been collected as a secondary D:A:D endpoint . All prospectively documented cases of DM were verified by the submission of a D:A:D event monitoring case report form. New onset DM was defined as either *definite* diagnosis if fasting plasma glucose >7.0 mmol/L (126 mg/dl) was measured on two consecutive occasions, or *possible* diagnosis in case of physician reported date of DM onset and initiation of anti-diabetic therapy.

Statistical methods. The rate of new onset DM was defined as the number of cases of DM divided by the total person-years of follow-up (PYFU)). PYFU were counted in a similar way to the primary analyses of the D:A:D Study (1) from the date of enrolment to the date of a first diagnosis of DM, death, 1st February 2006 or six months after the patient's last clinic visit, whichever occurred first. Factors associated with new onset DM were identified using Poisson regression models. We assessed the univariable relationships between duration of exposure to cART (defined as any

combination including a PI and/or non-nucleoside reverse transcriptase inhibitor [NNRTI]) and the rate of new onset DM. We then investigated whether any identified relationship applied to all drugs similarly, or whether it varied according to the type of antiretroviral drug received. These analyses were then adjusted to take account of possible demographic and clinical risk factors for DM, including age (fitted as a time-updated covariate), gender , transmission group (homosexual, injecting drug use, heterosexual, other/not known), race (white, black, other, not known), body mass index (BMI, categorized as <18, 18-26, 26.1-30 and >30 kg/m^2), smoking status (current, ex-, never and not known), patient's nadir CD4 count, duration of HIV infection prior to enrolment in D:A:D and calendar year. We further adjusted the analyses to take account of changes in lipids (total cholesterol, HDL-cholesterol and log_2-transformed triglycerides) and development of fat loss (lipoatrophy) or fat gain (lipohypertrophy) . Each of these variables was included as a time-updated covariate in a separate multivariable model with treatment exposure and other demographic/clinical risk factors. All analyses were adjusted for cohort.

RESULTS

Overall, 952 of the 33,389 patients in D:A:D had a diagnosis of DM at entry to the study, giving a baseline prevalence of 2.85% (95% confidence interval [CI]: 2.67-3.03). The characteristics of the remaining 32,437 patients are shown in Table 1. In these patients, over 130,151 PYFU, 744 patients were diagnosed with DM incidence of 5.72 per 1,000 PYFU (95% CI: 5.31-6.13)- 474 (63.7%) being definite and 270 (36.3%) possible diagnoses.

The incidence of new onset DM increased with cumulative exposure to cART (Figure 1(i)). This was significant in univariable analyses (unadjusted relative rate per year of exposure to cART: 1.06 [95% CI 1.03-1.09], p=0.0001), and after adjustment for other potential risk factors for DM (1.11 [1.07-1.15]; p=0.0001).

When analyzing whether the relationship with drug exposure was similar for all antiretroviral drugs, several findings emerged (Table 2). The strongest relationship with new onset DM was with exposure to stavudine (adjusted RR per year of exposure: 1.19 [1.15-1.24]; p=0.0001, unadjusted rates are shown in Figure 1(ii)). Whilst exposure to zidovudine and didanosine were also associated with an increased risk of new onset DM, exposure to ritonavir and nevirapine were both associated with a reduced risk . No other antiretroviral drug was significantly associated with the incidence of DM after adjusting for exposure to these drugs. Other demographic and clinical factors associated with increased risk of new onset DM were older age, male sex, greater BMI, heterosexual or IDU risk group, black African and other ethnicities and earlier calendar year. Current cigarette smoking was associated with a reduced risk . After adjusting for these factors, there were only weak and non-significant relationships between new onset DM and the patient's nadir CD4 count (RR per 50 cells/mm^3 higher: 0.98 [0.96-1.00]; p=0.06) and duration of HIV infection at enrolment in D:A:D (RR per additional year: 0.98 [0.96-1.00]; p=0.09).

In a series of multivariable analyses adjusted for these demographic and clinical factors as well as exposure to the five drugs, time-updated total cholesterol, HDL-cholesterol and lipodystrophy - either peripheral loss or central fat gain - were associated with new onset DM. A one mmol/L higher total cholesterol level was associated with a 9% increased rate of DM (RR: 1.09 [1.03-1.15]; p=0.001), a one mmol/L higher HDL-cholesterol with a 49% reduction in the rate of DM (0.51 [0.40-0.66]; p=0.0001), a two-fold higher triglyceride level with an 81% increase in DM rate (1.81 [1.67-1.95]; p=0.0001) and fat loss or fat gain with 28% and 57% increases in the risk of DM respectively

(1.28 [1.04-1.57]; p=0.02 and 1.57 [1.28-1.92]; p=0.0001). Whilst it was not possible to fit models that included both total cholesterol and triglycerides, due to the correlation between the two, models that included fat loss/gain as well as HDL cholesterol and triglycerides (Table 3), confirmed that HDL cholesterol (0.75 per mmol/L higher [0.58-0.96]; p=0.02), triglycerides (1.64 per two-fold higher [1.50-1.80]; p=0.0001) and fat gain (1.36 [1.09-1.68]; p=0.006) (but not fat loss) were all independently associated with new onset DM. Adjustment for these variables did not substantially modify the relationships between the five drugs and DM, although the relationship with stavudine was reduced from 1.19 to 1.13.

CONCLUSIONS

Our results show a significant relationship between new onset DM and exposure to cART, this effect being mainly related to exposure to stavudine, but exposure to zidovudine and didanosine were also associated with an increased risk , whereas exposure to ritonavir and nevirapine were both associated with a reduced risk of DM. This is Our findings are consistent with two other cohort studies which showed a similar relationship between exposure to stavudine and incidence of DM (2,3). We found a lower incidence of new onset DM than in the Multicenter AIDS Cohort Study (MACS) - 5.72 per 1000 PYFU, versus 47 and 17 per 1000 PYFU among individuals with or without cART (2). This difference could be related to different size and demographic compositions of both cohorts - the MACS involved exclusively white males likely to be exposed to the typical North American diet, older and with a higher BMI than D:A:D participants. In addition, in the MACS a single elevated fasting blood glucose was sufficient to establish a diagnosis of DM, a less stringent criterium than used in our study. Importantly, in the MACS, fasting glucose levels were obtained as part of the assessments at predefined cohort visits, whereas in our cohort the validation of DM as an endpoint is dependent on the actual screening policy for glucose intolerance and DM which is in place in each of the 212 treatment units contributing data to D:A:D, which undoubtedly differs between centers.

The association between DM/insulin resistance and stavudine/zidovudine might be explained through an indirect mechanism, i.e lipoatrophy which is a state which is associated with insulin resistance. The increased lipolysis observed in patients with lipoatrophy actually reflects their adipose tissue being insulin resistant. Lipolysis leads to increased circulating free fatty acids which may reinforce insulin resistance in the liver and skeletal muscles (4).

However, clinical evidence for a direct effect of thymidine analogues nucleoside reverse transcriptase inhibitors (NRTIs) on insulin sensitivity is also emerging. ART-naïve subjects randomized to stavudine- and didanosine-based therapy had a significant increase in HOMA-IR at 1 month while there was no change in those randomized to abacavir and lamivudine (5). Exposure to stavudine and didanosine is associated with greater lipoatrophy, illustrating the link between drug-related lipoatrophy and insulin resistance (2-6). Stavudine exposure leads to depletion of mitochondrial DNA content which may result in mitochondrial dysfunction. A recent study performed in healthy volunteers demonstrated that a one-month exposure to stavudine reduces insulin sensitivity in parallel with a 58% reduction in muscle mitochondrial DNA suggesting that mitochondrial dysfunction and insulin sensitivity were linked (7). Several studies have shown that Data in HIV uninfected individuals have suggested that mitochondrial dysfunction precedes the onset of DM in insulin resistant offspring of patients with type 2 DM. Alterations of several genes involved in mitochondrial oxidative phosphorylation have been identified in muscle samples of patients with

type 2 DM and impaired glucose tolerance (8-10).

The other risk factors for DM identified in our study included male sex, older age, greater BMI and black race, largely consistent with other studies in both the HIV-uninfected and HIV-infected populations (11). Current smoking status appeared to be marginally protective, contradicting some studies but consistent with results from the MRFIT trial of interventions for the reduction of CVD (12). The lower incidence of DM in recent calendar years could be inherent to the study design, i.e. follow up of a closed cohort where the patients at risk (i.e. susceptible to develop an end point) experience this relatively soon after enrolment reducing the subsequent risk in the cohort. It is also possible that the movement away from "old" drugs, particularly stavudine, towards alternative agents which are not associated with lipoatrophy development and have less or no effect on mitochondria may partially explain this finding.

Our data do not show a significant relationship between cumulative exposure to PIs and new onset DM. This is in line with a recent study in PI-exposed women which showed that cumulative exposure to PI was not associared with incidence of DM while cumulative exposure to NRTI was. (3) Antiretroviral regimens that include PIs for the treatment of HIV-1 have been associated with new-onset DM and insulin resistance (13-14). Reversal of hyperglycemia after PI withdrawal, onset of hyperinsulinemia before measurable body composition changes in PI recipients, and improvements in insulin sensitivity after substitution of PIs by the NNRTI nevirapine or the NRTI abacavir all suggest a direct effect of PIs on reducing insulin sensitivity in HIV-infected patients (15-16). In our study, we focused on the cumulative effect of exposure to antiretroviral drugs. Data from other studies suggest that the effect of indinavir on insulin resistance is of acute onset rather than cumulative or long term effect, and that this effect is reversible following drug discontinuation The acute effect seen with indinavir in human volunteers on insulin resistance is limited in size and no way in order of magnitude of the relationship seen with lipodystrophy. The Swiss HIV Cohort Study recently showed that new onset DM was independently associated with current exposure to indinavir, lamivudine-stavudine, didanosine-stavudine and didanodine-tenofovir; other PI and NRTI also showed trends (17). In a preliminary analysis we tested whether current use of indinavir or other PIs were associated with the risk of developing DM, which did suggest that current indinavir exposure was an additional risk factor for DM in our dataset. Additional analyses are planned to quantify this effect. The apparent slightly protective effect of ritonavir should be viewed cautiously; it could reflect the increasing use of more recent ritonavir-boosted PI regimens with less impact on insulin sensitivity.

Total cholesterol, HDL cholesterol and triglycerides were all associated with new onset DM after adjustment for demographic and clinical factors as well as for stavudine exposure, but adjusting for lipid parameters only slightly reduced the relationship between stavudine and DM. This relationship could be due to a common pathophysiologic mechanism leading to both lipid disorders and DM. Alternatively, lipolysis and increased serum free fatty acids have been documented in HIV-positive individuals. Excess free fatty acids in the circulation may reduce insulin sensitivity through inappropriate lipid storage in muscle and liver, resulting in impaired glucose utilization and insulin-mediated inhibition of glucogenolysis and gluconeogenesis (6, 18-20).

Clinically observed lipodystrophy was also significantly associated with new onset DM in accordance with previous studies showing that abnormal body fat distribution in HIV-positive individuals is strongly associated with insulin resistance and/or glucose intolerance, excess trunk or visceral fat being, as in the general population , a

risk factor for insulin resistance among those with HIV infection. In addition, insulin resistance is itself independently associated with fat loss in HIV-positive individuals (4, 21)

Our data confirm previous findings on the relationship between new onset of DM and exposure to stavudine (but also zidovudine and didanosine), increased total cholesterol, decreased HDL cholesterol and increased triglycerides. Interestingly, relationships for those parameters remained significant after adjustment for all other available risk factors. The large size of this cohort provides greater power to detect findings that other cohorts may be insufficiently powered to detect. However, it is possible that some of these results may reflect chance findings. There are several limitations of the study that should be considered: firstly, cohort studies such as ours cannot formally determine causality. However, they do permit an association between drug exposure and incidence of DM to be established and our findings are both consistent with other cohorts and biologically plausible. Secondly, factors such as treatment interruptions or changes in adherence are not taken into account because of the complex treatment patterns in this population. Finally, whilst we recognize that standardized assessments of lipodystrophy would have been preferable, this is unrealistic to achieve in a large multi-cohort collaboration such as this.

The relationship between new onset DM and exposure to stavudine (and other NRTIs) and lipodystrophy is a striking finding, particularly as adjustment for lipodystrophy did not modify the relationship between stavudine and DM. It is plausible that stavudine and other NRTIs directly contribute to insulin resistance and DM, apart from any indirect effect by way of lipodystrophy development. It should also be noted that our binary categorization of lipodystrophy may provide a relatively blunt tool with which to perform statistical adjustment; adjustment for the degree to which lipodystrophy is present (rather than just its presence or absence) may explain a higher proportion of the effect of stavudine. However, this level of information on lipodystrophy is rarely collected.

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TABLE 1. Characteristics of patients at enrolment in the D:A:D study

		n	(%)
Number of patients included		32437	(100.0)
Sex :	Male	23945	(73.8)
Age (years)	Median (IQR)	38	(33-44)
Mode of infection	Homosexual	13931	(43.0)
	Injecting drug use	5849	(18.0)
	Heterosexual	9759	(30.1)
	Other/not known	2898	(8.9)
Race:	White	14492	(44.7)
	Black	3334	(10.3)
	Other	952	(2.9)
	Not known	13659	(42.1)
Current smoker:		10885	(33.6)
Ever smoked:		15620	(48.2)
Body mass index (Kg/m^2):	Median (IQR)	23.0	(21.0-25.2)
CD4 count at entry ($cells/mm^3$):	Median (IQR)	410	(250-600)
Duration of injection (yrs):	Median (IQR)	5.0	(1.6-9.8)
Previous AIDS:		7832	(24.2)
Treatment status et entry:			
Antiretroviral-naïve:		8778	(27.1)
Use of any ARV :Median (IQR) exposure duration 3.1 (1.6,4.8) yrs		23660	(72,9%)
Use of any PI : Median (IQR) exposure duration 2.3 1.2,3.2) yrs		18751	(57,8%)
Use of any NNRTI: Median (IDR) exposure duration: 0.9 (0.4,1.6) yrs		10705	(33,0%)

TABLE 2. Results from multivariable analyses of demographic and clinical factors associated with new onset DM

		Relative rate	95% CI	p-value
Exposure to antiretroviral drugs (per yr)				
Stavudine		1.19	1.15-1.24	0.0001
	Zidovudine	1.06	1.03-1.10	0.0004
	Didanosine	1.06	1.02-1.11	0.01
	Ritonavir	0.94	0.89-0.99	0.01
	Nevirapine	0.89	0.84-0.95	0.0001
Age (per 5 years)		1.28	1.24-1.32	0.0001
Male sex		1.61	1.30-2.00	0.0001
BMI	<18	0.73	0.41-1.31	
	18-26	1	-	
	26-30	2.17	1.79-2.63	
	>30	4.47	3.53-5.67	
	Not known	1.18	0.95-1.46	0.0001
Ethnicity	White	1	-	
	Black	1.85	1.36-2.53	
	Other	1.87	1.21-2.88	
	Not know	1.28	0.75-2.19	0.0005
Risk group	Homosexual	1	-	
	Heterosexual	1.45	1.15-1.82	
	IDU	1.21	0.98-1.48	
	Other risk	1.10	0.83-1.44	0.02
Smoking status	Current smoker	0.76	0.62-0.93	
	Ex-smoker	0.98	0.79-1.20	
	Never smoker	1	-	
	Not known	0.90	0.67-1.20	0.04
Year	199/2000	3.40	2.52-4.60	
	2001	2.60	1.96-3.45	
	2002	1.85	1.39-2.47	
	2003	2.22	1.69-2.91	
	2004	1.37	1.03-1.83	
	2005/6	1	-	0.0001

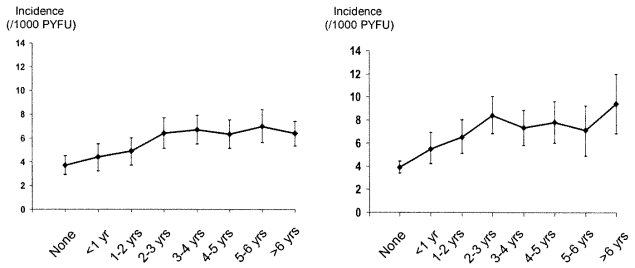
* All results are also adjusted for all variables and for cohort

TABLE 3. Results from multivariable analyses* to assess the association between exposure to five antiretroviral drugs and new onset DM after adjusting for time-updated metabolic parameters**

		Relative rate	95% CI	p-value
Exposure to antiretroviral drugs (per yr)				
Stavudine		1.13	1.08-1.15	0.0001
	Zidovudine	1.05	1.01-1.10	0.01
	Didanosine	1.06	1.01-1.11	0.02
	Ritonavir	0.90	0.85-0.95	0.0001
	Nevirapine	0.92	0.86-0.99	0.02
HDL cholesterol	(per mmol/L higher)	0.75	0.58-0.96	0.02
Triglycerides	(per $\log_2$ higher)	1.64	1.50-1.80	0.0001
Fat loss		1.09	0.88-1.36	0.42
Fat gain		1.36	1.09-1.68	0.006

- Estimates also adjusted for all variables shown in Table 2
- **Relationship with each drug was adjusted for all others.

Figure 1: Rates (per 1000 PYFU) of new onset DM stratified by years of exposure to (i) cART and (ii) stavudine



i) Exposure to cART **ii) Exposure to stavudine**